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ORIGINAL ARTICLE

Donor tobacco smoking is associated with postoperative thrombosis after primary liver transplantation

Yanni Li[1,2] | Lianne M. Nieuwenhuis[3] | Maureen J. M. Werner[3] | Michiel D. Voskuil[1,2] | Ranko Gacesa[1,2] | Hans Blokzijl[1] | Ton Lisman[3] | Rinse K. Weersma[1] | Robert J. Porte[3] | Eleonora A. M. Festen[1,2] | Vincent E. de Meijer[3]

[1]Department of Gastroenterology and Hepatology, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands

[2]Department of Genetics, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands

[3]Department of Surgery, Section of Hepatobiliary Surgery and Liver Transplantation, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands

Correspondence
Eleonora A. M. Festen, Department of Gastroenterology and Hepatology, University Medical Center Groningen, PO Box 30.001, 9700RB, Groningen, the Netherlands.
Email: e.a.m.festen@umcg.nl

Vincent E. de Meijer, Department of Hepatobiliary Surgery and Liver Transplantation, University Medical Center Groningen, Hanzeplein 1 – Ba33, 9700 RB, Groningen, the Netherlands.
Email: v.e.de.meijer@umcg.nl

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Abstract

Background: Thrombosis after liver transplantation is a leading cause of graft loss, morbidity, and mortality. Several known recipient- and surgery-related characteristics have been associated with increased risk of thrombosis after transplantation. Potential donor-related risk factors, however, remain largely undefined.

Objectives: We aimed to identify risk factors for early post-transplantation thrombosis (<90 days) and to determine the impact of early postoperative thrombosis on long-term graft and patient survival.

Patients/Methods: A post hoc analysis was performed of an observational cohort study including all primary, adult liver transplantations performed between 1993 and 2018. Donor-, recipient-, and surgery-related characteristics were collected. Competing risk model analyses and multivariable regression analyses were performed to identify risk factors for developing early post-transplant thrombosis and graft failure.

Results: From a total of 748 adult liver transplantations, 58 recipients (7.8%) developed a thrombosis after a median of 7 days. Post-transplantation thrombotic events included 25 hepatic artery thromboses, 13 portal vein thromboses, and 22 other thrombotic complications. Donor history of smoking was independently associated with early postoperative thrombosis (odds ratio [OR] 2.42; 95% confidence interval [CI], 1.29-4.52). Development of early post-transplant thrombosis was independently associated with patient mortality (hazard ratio [HR] 3.61; 95% CI 1.54-8.46) and graft failure (HR 5.80, 95% CI 3.26-10.33), respectively.

Conclusion: Donor history of smoking conveys a more than two-fold increased risk of thrombosis after liver transplantation, independent of other factors. Post-transplant thrombosis was independently associated with decreased patient and graft survival.

KEYWORDS

graft survival, hepatic artery thrombosis, liver transplantation, portal vein thrombosis, risk factors

Yanni Li and Lianne M. Nieuwenhuis contributed equally to this study.

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1 | INTRODUCTION

Postoperative thrombosis is a potentially life-threatening complication for orthotopic liver transplantation (OLT) recipients, which impairs graft survival and contributes significantly to adverse outcomes.[1,2] Studies in both pediatric and adult cohorts estimate an incidence of thrombotic events in up to 26% of cases. The most common types of thrombosis after liver transplantation are hepatic artery thrombosis (HAT) and portal vein thrombosis (PVT), which are reported at rates from 2% to 9% and 3% to 15%, respectively.[3-6]

Previous studies have suggested that, in addition to technical difficulties during surgery, donor/recipient size and immunological mismatches, rejection, re-transplantations, and specific donor characteristics such as age or weight (summarized in Table S1 in supporting information) can lead to an increased risk of early HAT or PVT.[7-9] The exact mechanism is unknown; however, dysregulated hemostasis and liver disease-related hypercoagulation have been proposed to play a substantial role.[10,11] Patients with a liver disease can present with hemostasis-related bleeding episodes, but may also be at risk for developing thromboembolic complications. The hemostatic capacity of patients with liver disease appears to be more easily disturbed compared to healthy individuals, which leads to a "rebalanced hemostasis" in the early phase of a post liver transplant patient.[12]

Therefore, the aim of this study was to identify which of these donor, recipient, and surgical characteristics are risk factors for early postoperative thrombosis in adult OLT recipients, and additionally to evaluate the effect of early postoperative thrombosis on short- and long-term graft survival and patient mortality.

Essentials

- Little is known about donor-related risk factors for developing post-transplant thrombosis.
- An observational study including primary adult liver transplantations between 1993-2018.
- A significantly higher incidence of graft loss was found in patients with thrombosis.
- Donor history of smoking was associated with an increased risk of post-transplant thrombosis.

2 | METHODS

2.1 | Study design and patients

A post hoc analysis of an observational cohort study of adult patients, who underwent a primary OLT at the University Medical Center Groningen, the Netherlands, between January 1993 and February 2018 was performed. Adult (age ≥ 18 years) OLT recipients with clinical recording and follow-up data were included in the study cohort. Only primary liver transplantations were included in our study (Figure 1). Heparin was not routinely administered perioperatively. All patients in our study received daily subcutaneous injections of Fraxiparine dosed at 2850 IU, starting at 6 hours post-transplantation. We collected all necessary data from existing databases and patient records, with a follow-up period until May 2018. Patient information before and during transplantation, 3 months after transplantation, and until end of follow-up period

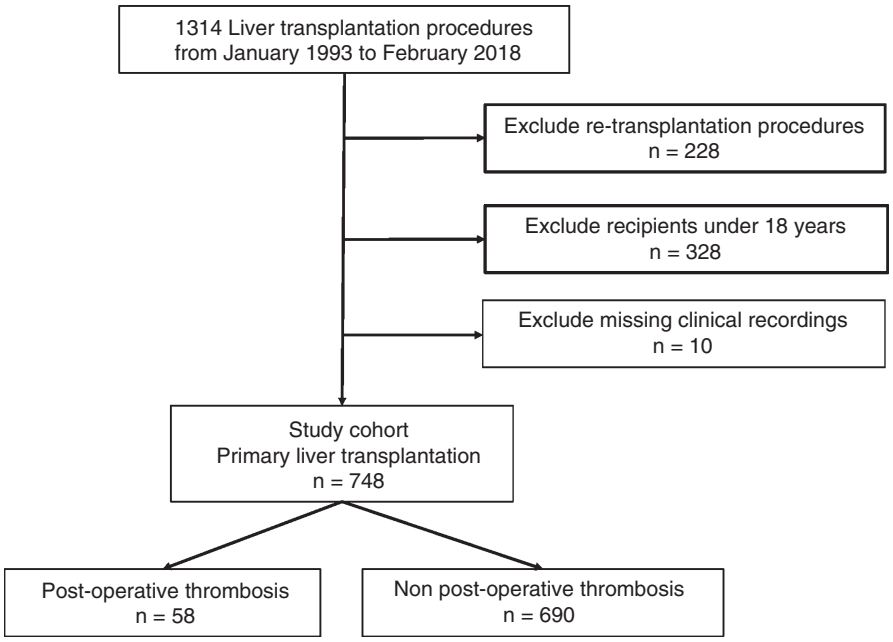


FIGURE 1 Consort diagram of patients included in the study. Flow diagram showing inclusion/exclusion and follow-up period

was collected. In addition, phenotypic and biochemical data of the donors was collected. The observational cohort study was registered in the Netherlands Trial Register (www.trialregister.nl; Trial NL6334) and was conducted within the TransplantLines cohort (TransplantLines; METc 2014/77). STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines for reporting observational studies were adhered to.[13]

2.2 | Definitions and variables

Early postoperative thrombosis was defined as any thrombotic event within the first 90 days after primary OLT. A thrombotic event was defined as a thrombus which was clinically suspected after OLT and later confirmed intraoperatively or with clinical imaging (ultrasound/angiography/computed tomography). Thrombotic events included HAT, PVT, and other postoperative vascular complications like pulmonary embolism, deep vein thrombosis, and venous outflow tract obstruction. HAT was defined as radiologically or surgically proven thrombosis of the hepatic artery. PVT was defined as radiologically or surgically proven thrombosis of the portal vein.

Patient survival was defined as time from OLT to death or end of follow-up (censored at 10 years after baseline or on May 1, 2018). Graft survival was defined as time between date of OLT and date of graft failure, death, or end of follow-up.

2.3 | Statistical analysis

Continuous data were presented as medians with interquartile ranges (IQR), and categorical data were presented as number (percentages). Mann-Whitney U and Pearson chi-square tests were used to test for differences in continuous and categorical variables, respectively. We compared all relevant donor, recipient, and surgical variables between the HAT, PVT, and all thrombotic events group versus non-thrombotic OLT recipients. Variables with a *P*-value < .1 in the univariable analysis, with valid data of more than 80%, were included in the multivariable analysis and were regarded as possible confounders and included in the risk models. Multivariable logistic regression analysis using a forward stepwise selection method was used to examine the independent association between candidate factors and the occurrence of thrombosis. In model 1, we performed a crude regression analysis. In model 2, we cumulatively adjusted for significant recipient variables in the univariable analysis ($P < .1$). In model 3, we cumulatively adjusted for the significant donor variables. In model 4, we cumulatively adjusted for the significant surgical variables. In model 5, we cumulatively adjusted for the other significant variables. Finally, in model 6 we cumulatively adjusted for potential confounders that were reported in previous studies. Results were presented as odds ratios (ORs) with corresponding 95% confidence intervals (CIs).

A competing risk survival analysis was performed within the dataset to evaluate patient and graft survival, which is an alternative method for analyzing competing risks of graft survival and patient death. The outcomes "patient death" and "graft failure" were included in the competing risk model. Cox regression analysis was used to compare graft survival and patient mortality in thrombosis and non-thrombosis groups. Cox proportional hazard models were constructed to adjust for potential confounders. All reported *P*-values are two-tailed and considered statistically significant if $P < .05$. Statistical analyses were conducted with the use of SPSS statistical software (IBM Corp, release 2015, version 23.0) and R software (3.5.3).

3 | RESULTS

3.1 | Baseline characteristics

We analyzed 748 adult patients who underwent a primary OLT for a variety of indications. Donor, recipient, and surgical characteristics are shown in Table 1. After a median (IQR) of $7^{4\text{-}22}$ days after OLT, 58 of 748 (7.8%) patients developed postoperative thrombosis. HAT occurred in 25/58 patients (time to event, 6 [2-20] days), PVT in 13/58 patients (time to event, 6 [4-15] days), and 22/58 (time to event, 14 [6-26] days) patients experienced other postoperative thrombotic complications. Total hospital- and intensive care unit (ICU) stay were significantly longer in the group of patients with any postoperative thrombotic event. Furthermore, the year of OLT was not significantly different between thrombosis and non-thrombosis groups.

3.2 | Risk factors for post-transplant thrombotic complications

We compared all relevant donor, recipient, and surgical variables between the HAT, PVT, and all thrombotic events group versus non-thrombotic OLT recipients. To obtain sufficient statistical power, we combined transplantation- and liver-specific thrombotic events (HAT and PVT). Parameters with a *P*-value < .1 in the univariable analyses were used for further multivariable regression analyses. Thus, cytomegalovirus (CMV) positive donor status (D+), CMV donor/recipient mismatch status (D+/R−), and donor history of smoking were included in the multivariable analysis for all postoperative thromboses. Recipient age, donor body mass index (BMI), donor history of smoking, partial graft and warm ischemia time (WIT) were included in the multivariable analysis for HAT and PVT. D+/R− CMV status and donor history of smoking were included in the multivariable analysis for other thrombotic events.

Univariable and multivariable regression analyses were performed for the HAT/PVT group, the other thrombosis group, and the combined group of postoperative thrombotic events. Odds ratios for the tested variables are summarized in Table 2 and Figure 2. The univariable analysis shows an association between donor history of smoking and the development of any thrombotic event (OR, 2.42; 95% CI, 1.29-4.52). In subgroup analyses, donor history of smoking

TABLE 1 Baseline characteristics of adult liver transplantation recipients with and without postoperative thrombosis

	Total		No thrombotic events		Post-transplantation thrombotic events			HAT + PVT subgroup			Other thrombosis subgroup		
	N = 748		N = 690		N = 58		*P*-value	N = 36		*P*-value	N = 22		*P*-value
Recipient characteristics													
Gender, male	433	(57.9)	401	(58.1)	32	(55.2)	.663	20	(55.6)	.762	12	(54.5)	.738
Time since OLT, years	12.5	(6.3-18.8)	12.4	(6.1-18.7)	13.8	(8.4-19.2)	.968	16	(11.1-20.9)	.411	15	(12.0-18.0)	.259
Age, years	51.2	(40.1-58.5)	49.9	(40.8-59.1)	51.8	(40.3-63.3)	.407	46.3	(34.8-57.8)	.038	56.5	(53.0-60.0)	.193
Weight, kg	76	(65.0-86.0)	74	(64.0-84.0)	73	(61.3-84.7)	.832	69	(57.1-80.9)	.448	77	(64.6-89.4)	.526
BMI, kg/m^2	24.9	(22.4-27.8)	24.2	(21.6-26.8)	25.2	(23.3-27.2)	.782	25.5	(23.6-27.4)	.715	24.5	(23.4-25.6)	.989
Indication													
Biliary cirrhosis	243	(32.5)	226	(32.8)	17	(29.3)	.441	10	(27.8)	.802	7	(31.8)	.439
Metabolic	128	(17.1)	116	(16.8)	12	(20.7)		8	(22.2)		4	(18.2)	
Post-Alcoholic	101	(13.5)	93	(13.5)	8	(13.8)		5	(13.9)		3	(13.6)	
Toxic/ Hepatitis	121	(16.2)	116	(16.8)	5	(8.6)		4	(11.1)		1	(4.5)	
NASH	46	(6.2)	43	(6.2)	3	(5.2)		2	(5.6)		1	(4.5)	
Others	108	(14.5)	95	(16.8)	13	(22.4)		7	(19.4)		6	(27.3)	
CP-score	9	(7-11)	9	(7-11)	8	(7-11)	.442	8	(4-12)	.672	9	(6-12)	.445
MELD score	16	(11-23)	15	(9-21)	15	(10-20)	.508	15	(10-20)	.563	15	(9-22)	.722
Malignancy	57	(10.5)	51	(7.5)	6	(10.5)	.415	3	(8.3)	.858	3	(14.3)	.253
Thrombosis in past	114	(15.3)	107	(15.5)	7	(12.3)	.515	4	(11.4)	.513	3	(13.6)	.811
High urgency	40	(5.3)	38	(5.5)	2	(3.4)	.503	2	(5.6)	.99	0	(0)	.258
CMV R + status	437	(61.7)	405	(62.1)	32	(57.1)	.462	20	(57.1)	.555	12	(57.1)	.644
CMV D+/R− status	117	(16.7)	103	(15.9)	14	(25.5)	.069	8	(22.9)	.281	6	(30)	.094
Hypertension in past	284	(54.3)	268	(54.8)	16	(47.1)	.381	6	(33.3)	.073[a]	10	(62.5)	.543
Smoking in past	114	(26.8)	102	(14.8)	12	(42.9)	.048[a]	8	(57.1)	.009[a]	4	(28.6)	.809
PNF	31	(5.2)	20	(3.6)	11	(22.9)	<.001	11	(40.7)	<.001	0	(0)	.373
Previous surgery	84	(24.9)	79	(25.6)	5	(17.9)	.367	4	(21.1)	.66	1	(11.1)	.325
Donor characteristics													
Donor age, years	46	(36.0-56.0)	46	(36.0-56.0)	47	(34.0-60.0)	.638	49	(33.5-64.5)	.977	42.5	(30.5-54.5)	.456
Gender, male	393	(52.5)	365	(52.9)	28	(48.3)	.498	18	(50)	.734	10	(45.5)	.491
Donor BMI, kg/m^2	24.5	(24.2-26.0)	24.2	(21.4-26.0)	24.2	(22.5-25.9)	.564	23.9	(22.4-25.4)	.064	25.1	(23.1-27.1)	.152
D + CMV status	340	(45.9)	307	(44.9)	33	(57.9)	.058	20	(55.6)	.21	13	(61.9)	.123

(Continues)

TABLE 1 (Continued)

	Total		No thrombotic events		Post-transplantation thrombotic events			HAT + PVT subgroup			Other thrombosis subgroup		
	N = 748		N = 690		N = 58		*P*-value	N = 36		*P*-value	N = 22		*P*-value
Donor Hypertension	166	(27)	154	(27.3)	12	(24)	.614	8	(28.6)	.883	4	(18.2)	.344
Donor smoker	317	(50.8)	282	(49.1)	35	(70)	.005	20	(69)	.037	15	(71.4)	.045
Donor alcohol abuse	37	(9.8)	32	(9.3)	5	(14.7)	.315	4	(22.2)	.075[a]	1	(6.3)	.677
Donor diabetes mellitus	27	(6.8)	23	(6.4)	4	(11.4)	.257	1	(5.6)	.89	3	(17.6)	.073[a]
Donor type													
Living donor	5	(0.7)	5	(0.7)	0	(0)	.646	0	(0)	.766	0	(0)	.837
DBD	617	(82.5)	567	(82.2)	50	(86.2)		31	(86.1)		19	(86.4)	
DCD	126	(16.8)	118	(17.1)	8	(13.8)		5	(13.9)		3	(13.6)	
Donor death reason													
External causes	192	(25.7)	175	(25.4)	17	(29.3)	.807	11	(30.6)	.625	6	(27.3)	.658
Cerebrovascular disease	528	(70.7)	489	(71)	39	(67.2)		23	(63.9)		16	(72.7)	
Others	27	(3.6)	25	(3.6)	2	(3.4)		2	(5.6)		0	(0)	
Graft type													
Partial	23	(3.1)	20	(2.9)	3	(5.3)	.333	3	(8.3)	.074	0	(0)	.425
Full size	714	(96.9)	660	(97.1)	54	(94.7)		33	(91.7)		21	(100)	
Surgical factors													
Surgical technique													
Piggyback	410	(68.9)	375	(68.6)	35	(72.9)	.531	23	(74.2)	.51	12	(70.6)	.859
Classical	185	(31.1)	172	(31.4)	13	(27.1)		8	(25.8)		5	(29.4)	
Biliary Anastomoses D-D	529	(87.3)	495	(87.6)	34	(82.9)	.385	22	(84.6)	.652	12	(80)	.38
CIT, min	498	(386-610)	500	(389-511)	488	(383-593)	.557	518	(398-637)	.256	472	(421-523)	.608
WIT, min	50	(31-69)	50	(40-59)	50	(40-60)	.296	49	(39-59)	.322	51	(40-62)	.648
Operation time, min	581	(493-668)	584	(498-672)	576	(489-669)	.262	555	(475-635)	.387	620	(523-717)	.061[a]
Blood loss, mL/kg	49.1	(13.7-84.6)	54.7	(13.2-96.2)	55.7	(8.7-102.1)	.339	78	(2.9-158.9)	.166	37.5	(1.5-53.5)	.837
Hospitalization, days	29	(17-40)	29	(17-40)	33	(20-47)	.022	26	(14-38)	.385	38	(28-48)	.007
ICU stays, days	3	(1-5)	3	(1-6)	4	(2-7)	.001	4	(2-6)	.029	5	(0-10)	.013

Note: Data presented as number (%) or median (interquartile range) when appropriate. *P*-value was calculated using Mann-Whitney U test and Pearson chi-square tests for differences in continuous and categorical variables, respectively.

Abbreviations: BMI, body mass index; CIT, cold ischemia time; CMV, cytomegalovirus; CP-score, Child Pugh-score; DBD, donation after brain death; DCD, donation after circulatory death; D-D, duct-duct; HAT, hepatic artery thrombosis; ICU, intensive care unit; MELD, model for end-stage liver disease; NASH, non-alcoholic steatohepatitis; PNF, primary non-function; PVT, portal vein thrombosis; WIT, warm ischemia time.

[a]Excluded for further analyses because of <80% valid data.

remained associated with liver transplant specific (ie, HAT and PVT) thrombosis (OR, 2.30; 95% CI, 1.03-5.14) as well as with other thrombotic events (OR, 2.59; 95% CI, 0.99-6.77). The variables with a *P*-value < .1 were selected for further multivariable analysis.

In the multivariable analysis (Figure 2, Table S2 in supporting information), donor history of smoking was identified as an independent predictor for developing any thrombotic event (OR 2.34; 95% CI, 1.23-4.45), as well as for developing HAT or PVT (OR 2.29; 95% CI, 1.02-5.13).

3.3 | Donor smoking as a risk factor for post-transplant thrombosis

To identify donor smoking as an independent risk factor for early postoperative thrombosis, we adjusted for potential confounders identified in previous literature (Table S1) and variables from the univariable regression analysis (Table 2). We found that donor history of smoking significantly increased the risk of all-cause thrombosis more than two-fold (Table 3, model 1: OR, 2.42; 95% CI, 1.29-4.52), when compared to non-smoking donors.

After adjusting for recipient-, donor-, and surgical-related factors and for previously reported confounders, donor history of smoking remained independently associated with postoperative thrombosis (Table 3, models 2-6).

The incidence of post-transplant thrombosis in recipients who received a liver from a donor without a history of smoking was 4.9%. In patients who received a liver from a donor with a history of smoking, the incidence of thrombosis increased to 11.0%.

3.4 | Patient and graft survival

Postoperative thrombosis had a substantial effect on graft and patient survival. Of all patients who developed postoperative thrombosis, a total of 62.1% experienced graft failure after a median follow-up period of 14 years, compared to 43.2% of people without postoperative thrombosis ($P = .005$). Causes for patient mortality include infection (19.4%), any thrombotic event (3.9%), rejection (1.6%), primary non-function (PNF) (1.6%), and trauma (0.3%). Figure 3 shows cumulative incidence functions for the progression to graft failure and patient death in HAT, PVT, total postoperative thrombosis, and patients without thrombosis groups. Competing risk analysis within the categories of postoperative thrombosis showed that both HAT and PVT had a significant effect on early graft survival, and that especially patients with HAT were more likely to experience graft failure in the first 90 days (log-rank, $P < .001$; Figure 3A). In addition, development of PVT was associated with increased mortality within the first 90 days post-transplantation (log-rank, $P = .026$; Figure 3A). Ten-year competing risk analysis was performed using data from recipients who had not experienced graft failure in the first 90 days. The analysis showed no significant difference in long-term patient and graft survival between all groups of thrombosis and without thrombosis for those who survived the first 90 days after transplantation (log-rank, $P = .35$ and $P = .32$; Figure 3B).

In the Cox regression analysis, development of early post-transplant thrombosis was significantly associated with patient mortality and graft failure with a hazard ratio [HR] of 3.61 (95% CI, 1.54-8.46) and 5.80 (95% CI, 3.26-10.33), respectively, when adjusted for potential confounders including age, D+/R- CMV status, donor history of smoking, donor BMI, graft type, WIT, and ICU and hospitalization time.

4 | DISCUSSION

This study aimed to identify risk factors for thrombosis after OLT. Donor, recipient, and surgical parameters were collected and compared between groups of patients with or without postoperative thrombotic complications. We have newly identified donor history of smoking as an independent risk factor for developing thrombosis after OLT. In addition, we show that postoperative thrombotic complications mainly affect graft survival in the early postoperative phase (first 90 days), rather than long-term graft survival (Figure 3).

In this study, we confirmed previously reported risk factors, and identified new risk factors for early postoperative thrombotic complications. We initially analyzed HAT and PVT separately as transplantation-specific thrombotic complications. As Table 2 shows, recipient age, donor BMI, donor history of smoking, and partial graft all were identified as potential risk factors for developing HAT/PVT. In our multivariable analysis, we have identified donor history of smoking as a new risk factor for developing any thrombotic event. Donor history of smoking was also confirmed as a risk factor for developing liver-specific thrombosis, HAT, and PVT. In addition, of the previously reported risk factors for post OLT thrombosis, lower recipient age and low donor BMI were confirmed for HAT and PVT in the univariable analysis.[14] This information is important when considering, for example, a liver graft from a donor with a low BMI and a history of smoking for a young recipient because of the increased risk of developing early postoperative thrombosis (Figure 2).

OLT candidates are strongly advised to quit smoking to decrease the risk of developing post-transplant thrombotic complications, as previous studies have identified a history of smoking in the recipient as an important risk factor.[15] The effect of donor history of smoking has been found to increase patient mortality after HAT.[16,17] It is unknown how donor history of smoking causes thrombosis in the recipient. However, previous studies have reported pathways in which cigarette smoking causes endothelial damage, which could eventually lead to thrombosis. These studies have shown that cigarette smoke has a cytotoxic effect on endothelial cells, both in vitro and ex vivo.[18] In vitro studies with human umbilical vein endothelial cells and a variety of human and mammalian cell types have shown an increase in the rate of cell death after exposure to cigarette smoke.[19,20] Heavy smokers also experienced impaired endothelium-dependent,

TABLE 2 Univariable analysis of baseline characteristics of adult primary liver transplantation recipients with and without postoperative thrombosis

	All post-transplantation thrombotic events					HAT + PVT subgroup					Other thrombosis subgroup				
	N = 58		OR	95% CI	*P*-value	N = 36		OR	95% CI	*P*-value	N = 22		OR	95% CI	*P*-value
Gender, male	32	(55.2)	0.89	(0.52-1.52)	.663	20	(55.6)	0.9	(0.46-1.77)	.762	12	(54.5)	0.87	(0.37-2.03)	.739
Age, years	51.8	(40.3-63.3)	0.99	(0.97-1.01)	.37	46.3	(34.8-57.8)	0.97	(0.95-0.99)	.034	56.5	(53.0-60.0)	1.02	(0.99-1.06)	.2
Weight, kg	73	(61.3-84.7)	0.99	(0.98-1.01)	.733	69	(57.1-80.9)	0.99	(0.97-1.01)	.442	77	(64.6-89.4)	1.01	(0.98-1.03)	.666
BMI, kg/m^2	25.2	(23.3-27.2)	0.97	(0.91-1.04)	.359	25.5	(23.6-27.4)	0.97	(0.89-1.05)	.394	24.5	(23.4-25.6)	1	(0.91-1.10)	.997
Indication			-	(-)	.441			-	(-)	.802			-	(-)	.439
Biliary cirrhosis	17	(29.3)				10	(27.8)				7	(31.8)			
Metabolic	12	(20.7)				8	(22.2)				4	(18.2)			
Post-Alcoholic	8	(13.8)				5	(13.9)				3	(13.6)			
Toxic/ Hepatitis	5	(8.6)				4	(11.1)				1	(4.5)			
NASH	3	(5.2)				2	(5.6)				1	(4.5)			
Others	13	(22.4)				7	(19.4)				6	(27.3)			
CP-score	8	(7-11)	0.96	(0.86-1.08)	.497	8	(4-12)	0.98	(0.85-1.13)	.8	9	(6-12)	0.92	(0.75-1.12)	.396
MELD score	15	(10-20)	1.01	(0.98-1.04)	.532	15	(10-20)	1.01	(0.98-1.04)	.571	15	(9-22)	1.01	(0.97-1.05)	.755
CMV D+/R- status	14	(25.5)	1.8	(0.95-3.42)	.073	8	(22.9)	1.56	(0.69-3.54)	.284	6	(30)	2.26	(0.85-6.02)	.103
Donor age, years	47	(34.0-60.0)	1	(0.98-1.02)	.899	49	(33.5-64.5)	0.99	(0.97-1.02)	.664	42.5	(30.5-54.5)	1.01	(0.98-1.04)	.441
Donor BMI, kg/m^2	24.2	(22.5-25.9)	0.96	(0.89-1.04)	.351	23.9	(22.4-25.4)	0.9	(0.80-0.99)	.046	25.1	(23.1-27.1)	1.05	(0.95-1.16)	.331
D + CMV status	33	(57.9)	1.69	(0.98-2.92)	.06	20	(55.6)	1.54	(0.78-3.01)	.213	13	(61.9)	2	(0.82-4.88)	.13
Donor smoker	35	(70)	2.42	(1.29-4.52)	.006	20	(69)	2.3	(1.03-5.14)	.042	15	(71.4)	2.59	(0.99-6.77)	.052
Partial graft	3	(5.3)	1.83	(0.53-6.37)	.34	3	(8.3)	3	(0.85-10.6)	.088	0	(0)	-	(-)	.998
Biliary anastomoses Roux-Y	34	(82.9)	1.46	(0.62-3.41)	.385	22	(84.6)	1.29	(0.43-3.84)	.653	12	(80)	1.77	(0.48-6.42)	.387
CIT, min	488	(383-593)	1	(0.99-1.00)	.557	518	(398-637)	1	(0.99-1.00)	.3	472	(421-523)	0.99	(0.99-1.00)	.365
WIT, min	50	(40-60)	1.01	(0.99-1.03)	.1	49	(39-59)	1.02	(0.99-1.04)	.067	51	(40-62)	1.01	(0.98-1.03)	.717
Blood loss, mL/kg	55.7	(8.7-102.1)	1	(0.99-1.00)	.895	78	(2.9-158.9)	1	(0.99-1.00)	.583	37.5	(1.5-53.5)	0.99	(0.99-1.00)	.615
Hospitalization, days	33	(20-47)	1.01	(1.00-1.02)	.017	26	(14-38)	1.01	(0.99-1.02)	.233	38	(28-48)	1.01	(1.00-1.03)	.014
ICU stays, days	4	(2-7)	1	(0.99-1.02)	.46	4	(2-6)	0.99	(0.97-1.03)	.88	5	(0-10)	1.01	(0.99-1.03)	.179

Note: Data presented as number (%) or median (interquartile range) when appropriate, with their respective odds ratios with 95% confidence intervals and *P*-values.

Abbreviations: BMI, body mass index; CI, confidence interval; CIT, cold ischemia time; CMV, cytomegalovirus; CP-score, Child Pugh-score; HAT, hepatic artery thrombosis; ICU, intensive care unit; MELD, model for end-stage liver disease; NASH, non-alcoholic steatohepatitis; OR, odds ratio; PVT, portal vein thrombosis; WIT, warm ischemia time.

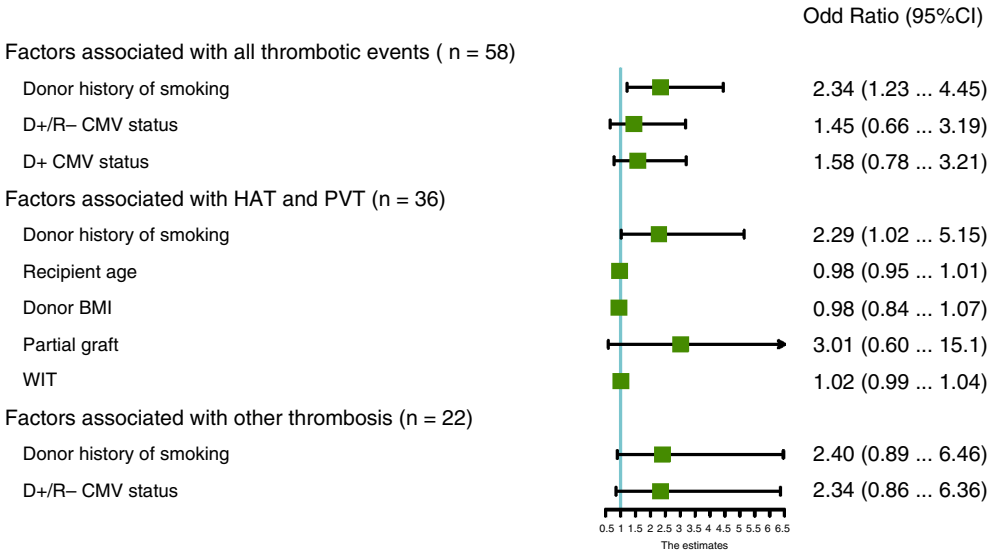


FIGURE 2 Multivariable analyses of risk factors in thrombotic subgroups. BMI, body mass index; CMV, cytomegalovirus; HAT, hepatic artery thrombosis; PVT, portal vein thrombosis. Note: Odds ratio represents the odds of thrombotic events with a risk factor compared to the odds of thrombotic events occurring in the absence of this risk

nitric oxide (NO) flow-mediated vasodilatation.[21] Additionally, Michaud et al reported that cigarette smoke exposure impairs vascular endothelial growth factor (VEGF)-induced endothelial cell migration and tube formation. This increases the generation of reactive oxygen species (ROS), decreases expression of surface integrins, and blocks the Akt/eNOS/NO pathway.[22] Our study has identified that a history of smoking in the donor was associated with increased risk of early postoperative thrombosis. In our patient cohort, donor cigarette smoking may be responsible for morphological and functional damage to the endothelium of the liver graft, which then may lead to thrombosis in the recipient. However, future research to confirm this pathway is needed.

An association between recipient CMV positive status and early postoperative thrombosis has already been shown in recent studies.[23] Our study confirmed the use of seropositive CMV grafts as a potential risk factor for developing early postoperative thrombosis. However, there is limited recent data on the effect of seropositive CMV grafts on developing thrombotic complications, with the latest

TABLE 3 Association of donor smokers with postoperative thrombosis of liver transplant

	No thrombosis	Overall postoperative thrombotic Group		HAT + PVT Group		Other thrombosis Group	
	Ref.	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value
No. of events	690	58		36		22	
Model 1	1.00	2.42 (1.29-4.52)	.006	2.30 (1.03-5.14)	.042	2.59 (0.99-6.77)	.052
Model 2	1.00	2.43 (1.30-4.54)	.006	2.33 (1.04-5.20)	.040	2.60 (0.99-6.79)	.052
Model 3	1.00	2.34 (1.23-4.44)	.010	2.23 (0.99-5.02)	.054	2.55 (0.94-6.91)	.066
Model 4	1.00	2.34 (1.25-4.41)	.008	2.27 (1.01-5.09)	.047	2.50 (0.94-6.61)	.065
Model 5	1.00	2.43 (1.26-4.67)	.008	2.44 (1.05-5.69)	.039	2.45 (0.92-6.53)	.073
Model 6*	1.00	4.48 (1.64-12.25)	.003	3.57 (1.06-11.97)	.040	6.70 (1.17-38.47)	.033

Note: Model 1: Donor history of smoking

Model 2: model 1 + adjustment for recipient age.

Model 3: model 1 + adjustment for CMV D+/R- status, donor BMI and donor CMV + status.

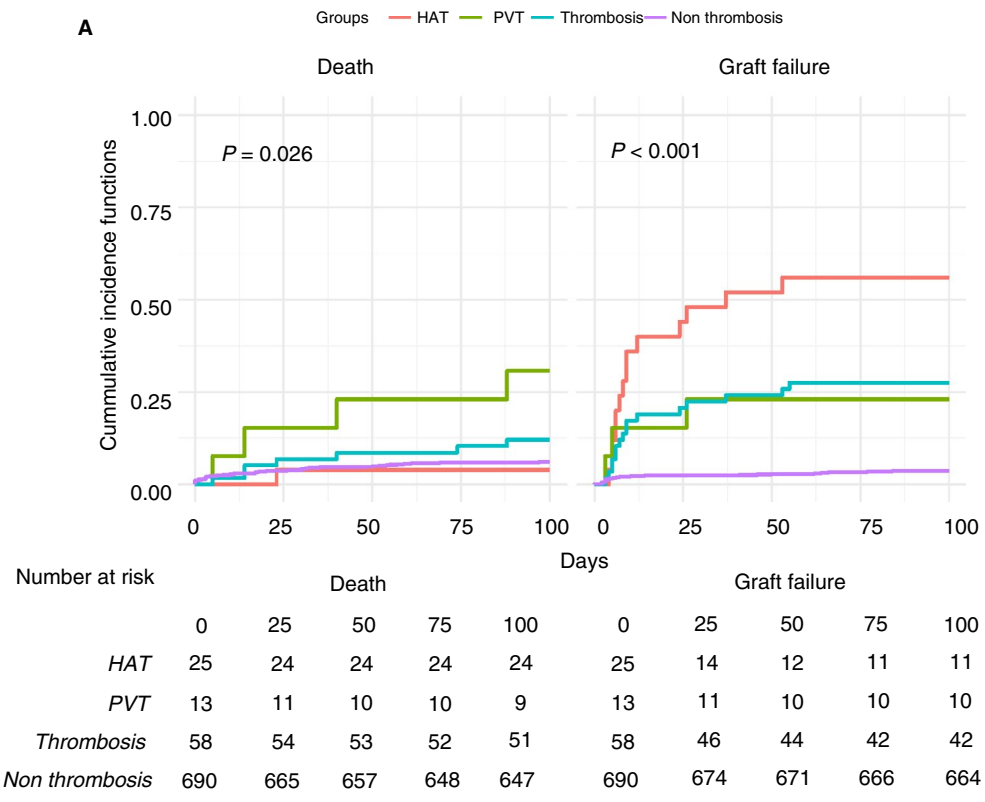
Model 4: model 1 + adjustment for WIT and partial graft type.

Model 5: model 1 + adjustment for hospitalization and ICU days.

Model 6: model 1 + adjustment for potential confounders which were reported in previous studies.

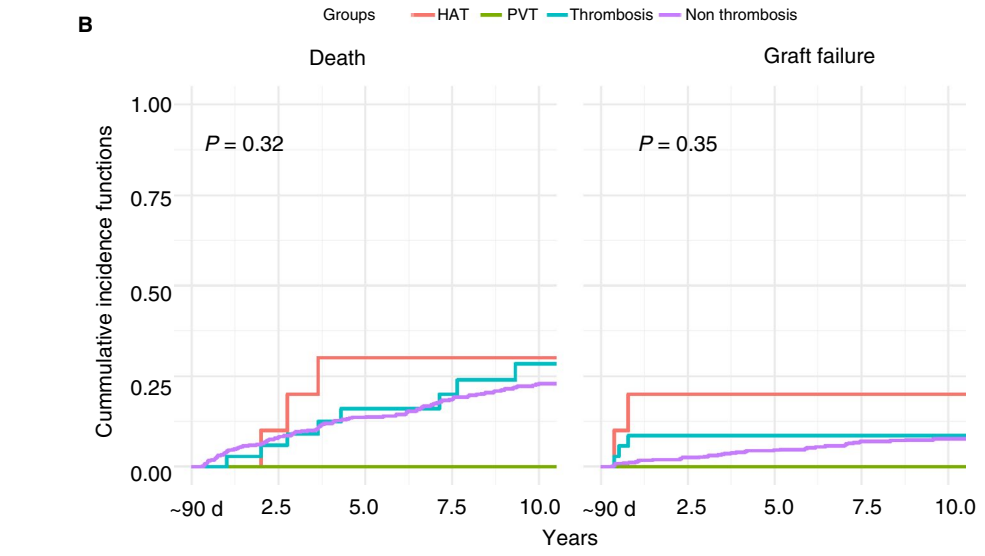
Abbreviations: BMI, body mass index; CI, confidence interval; CIT, cold ischemia time; CMV, cytomegalovirus; HAT, hepatic artery thrombosis; ICU, intensive care unit; MELD, model for end-stage liver disease; OR, odds ratio; PVT, portal vein thrombosis; WIT, warm ischemia time.

*Adjusted confounders: recipient gender, recipient body weight, recipient BMI, recipient transplant indication, Child-Pugh score, MELD score, donor age, Roux-en-Y biliary-reconstruction, CIT, and blood loss.



Number at risk

	Death					Graft failure				
	0	25	50	75	100	0	25	50	75	100
HAT	25	24	24	24	24	25	14	12	11	11
PVT	13	11	10	10	9	13	11	10	10	10
Thrombosis	58	54	53	52	51	58	46	44	42	42
Non thrombosis	690	665	657	648	647	690	674	671	666	664



Number at risk

	Death					Graft failure				
	~90 d	2.5 y	5 y	7.5 y	10 y	~90 d	2.5 y	5 y	7.5 y	10 y
HAT	10	9	7	6	5	10	8	8	7	6
PVT	6	4	3	3	3	6	4	3	3	3
Thrombosis	35	31	23	21	15	35	30	25	24	20
Non thrombosis	622	523	433	366	298	622	556	485	430	377

FIGURE 3 Cumulative incidence curves demonstrating the association between postoperative thrombosis groups and progression to graft failure or death in adult recipients for the first 90 days (A) and for 10 years (B) after liver transplantation

findings dating back to 2006.[24,25] Our results suggest an approximately 50% increase in the odds of any post-transplant thrombosis in patients with CMV D+ status or CMV D+/R− mismatch status. Nonetheless, odds ranging from a 34% decrease, a negative association, to a more than three-fold increase, a substantial positive association, is also reasonably compatible with our data, given our

assumptions. We hypothesize that this could, at least, partially be explained by CMV-mediated activation of endothelial cells, thereby interfering with donor hemostasis. The activated endothelial cells may subsequently promote liver-specific thrombosis in the recipient after OLT.[26]

The main limitation of this study is that because some variables had to be excluded from further analyses because of a relatively high number of missing values, it is possible that some important risk factors were missed. For example, major bleeding and blood product transfusions during transplantation might have an impact on thrombotic outcomes; however, they were not used in our analyses due a high number of missing values. Further research is needed to explore this association. Because in our data set the thrombotic events HAT and PVT occurred relatively infrequently (3.3% and 1.7%, respectively), we have combined them as a composite endpoint to gain statistical power. Although we acknowledge that HAT and PVT may have a different etiology when considering surgical and recipient risk factors, they are all specific to liver transplantation, and donor risk factors will likely contribute to all thrombotic events.

We included all OLTs performed during the last 25 years. As transplant care, including anesthesiology, surgery, intensive care, and post-transplant treatment, including immunosuppression, have substantially improved over time, we have investigated if the year of transplantation may confound our results. As mentioned before, we found no significant difference between year effect, indicating that the date of transplantation is unlikely to be a confounder. A strength of this study is the thorough investigation of the role of potential thrombosis risk factors, not only those reported previously. This has made it possible to identify new risk factors for postoperative thrombotic complications.

In conclusion, this study has identified donor history of smoking as an independent risk factor for developing thrombosis after OLT and has strengthened findings of previous studies. This study has also shown that the incidence of thrombosis leads to an increased incidence of graft failure within the first 90 days after transplantation. The selection of donor grafts can be further improved by considering smoking history, in addition to well-known recipient and surgical parameters. These results warrant future investigation into the contribution of donor risk factors for early thrombotic events of post OLT thrombosis.

CONFLICTS OF INTEREST

The authors of this manuscript have no conflicts of interest to disclose.

AUTHOR CONTRIBUTIONS

YL, LN, EF, and VM designed the study, interpreted results, and wrote the manuscript; MW and LN contributed to the collection of patient data; YL and LN conducted statistical analyses; MDV, TL, RW, and RP guided the interpretation of the results and research design. All authors critically revised the manuscript and approved the manuscript for publication.

ORCID

Yanni Li https://orcid.org/0000-0002-7021-9249
Maureen J. M. Werner https://orcid.org/0000-0002-5542-0286
Ton Lisman https://orcid.org/0000-0002-3503-7140
Vincent E. de Meijer https://orcid.org/0000-0002-7900-5917

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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